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MiniReview

The role of genome diversity and immune evasion in persistent infection with *Helicobacter pylori*

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Abstract

Helicobacter pylori is an important human pathogen that chronically colonizes the stomach of half the world's population. Infection typically occurs in childhood and persists for decades, if not for the lifetime of the host. How is bacterial persistence possible despite a vigorous innate and adaptive immune response? Here we describe the complex role of bacterial diversity and specific mechanisms to avoid or subvert host immunity in bacterial persistence. We suggest that *H. pylori* finely modulates the extent to which it interacts with the host in order to promote chronic infection, and that it uses diverse mechanisms to do so.

Keywords: *Helicobacter pylori*; Immune evasion; Genome diversity

1. Introduction

The normal human stomach was long considered sterile or colonized only transiently by small numbers of bacteria, owing to the bactericidal effects of the gastric acid barrier. The discovery of *Helicobacter pylori* in 1983 marked a turning point in our understanding of gastrointestinal microbial ecology and disease. It is now clear that chronic infection with *H. pylori* is the major cause of peptic ulcer disease and is an important factor in the development of gastric adenocarcinoma, the second most common cause of cancer mortality worldwide. Perhaps equally important from a biological standpoint is the growing appreciation that colonization of the stomach and intestinal tract with *Helicobacter* species is ubiquitous in the animal kingdom [1]. Like population of the gut with facultative and anaerobic microorganisms, gastric colonization with *Helicobacter* commonly occurs very early in life and in most cases has a predominantly commensal or perhaps symbiotic relationship with its host.

One of the early questions about the biology of *H. pylori* was similar to an enigma that has long puzzled gastroenterologists: how can the organism survive the harsh effects of gastric acid? The explanation is in large part that *H. pylori* and all other gastric *Helicobacter* species synthesize a potent urease that hydrolyzes urea and buffers the periplasm. But to persist in the stomach as it does, typically for the lifetime of the host, *H. pylori* must also overcome additional host defenses. These include aspects of innate defense, such as gastric peristalsis, antimicrobial factors (digestive enzymes, lactoferrin, and defensins, to name a few), gastric mucus, and epithelial cell turnover, as well as the adaptive host immune response.

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In view of its decades long colonization of more than half the world's population, *H. pylori* is arguably the single most successful bacterial pathogen of humans. In this review we discuss two important strategies that *H. pylori* exploits in order to promote chronic colonization. First, *H. pylori* isolates from unrelated individuals show marked genetic diversity that exceeds that seen with all known bacterial species. This diversity likely develops during the often lifelong association of the organism with its unique host, and reflects adaptation to host defense as well as other aspects of the changing gastric environment. Second, it has become increasingly clear that *H. pylori* has multiple means of immune suppression or avoidance, in effect staying below the radar screen of host defense. Of course, these categories are heuristic and not mutually exclusive. For example, diversity of surface proteins may provide a selective advantage by avoiding host immunity. Understanding these and other mechanisms of persistence will contribute not only to our understanding of *H. pylori* pathogenesis, but also has implications for biology and disease. For a broader discussion of persistence by *H. pylori* and other bacterial pathogens the reader is invited to consult excellent recent reviews [2,3].

2. *H. pylori* diversity

H. pylori is thought to be the most genetically diverse bacterial species studied to date. This genetic diversity is evidenced by a seemingly unlimited number of unique strains that differ in genome size, gene order, genetic content, and allelic profile. *H. pylori* diversity is clinically important because peptic ulcer disease and gastric cancer are more commonly associated with infection by some strains than by others, particularly those that contain the Cag pathogenicity island (PAI) and certain alleles of the VacA cytotoxin. Furthermore, *H. pylori* diversity has provided evidence for intrafamilial transmission and has even been used to trace ancient patterns of human migration [4].

Comparative analysis of the complete genome sequences of *H. pylori* strains 26695 and J99 has provided additional insight into the genetic diversity within *H. pylori*. Ten organizational changes are required to align the orthologous genes present in these two genomes. Although most genes are highly conserved between the two strains, ~6% of each genome encodes strain-specific genes. Further investigation of 15 *H. pylori* strains by whole-genome microarray analysis revealed that at least 12–18% of each strain's genome is composed of strain-specific genes. In addition, ~22% of the 1643 genes analyzed were missing from at least 1 of the 15 strains [5]. Many of these strain-specific genes are located within a highly variable region of the chromosome that has been termed the "plasticity zone" [6], which is believed to be a site of extensive insertion, excision, and recombination.

2.1. Mechanisms of diversification

Genetic diversity arises through mechanisms of point mutation, recombination, and genetic exchange, all of which are frequent in *H. pylori* (Fig. 1). *H. pylori* strains exhibit a wide range of mutation frequencies and hypermutable strains are common [7]. The lack of a *mutHLS*-like pathway for DNA mismatch repair may contribute to the high mutation frequency, but the possibility that *H. pylori* has an unidentified mismatch repair system cannot be excluded. Genetic diversity is also generated by recombination and lateral genetic exchange, which are so frequent in *H. pylori* that alleles at independent loci are rarely co-inherited for long periods of time and essentially exist in a state of linkage equilibrium. Due to such frequent genetic exchange, *H. pylori* strains isolated from unrelated individuals display a panmictic population structure and clonal descent is only discernable among strains of *H. pylori* isolated from family members [8]. Similar to other panmictic species such as *Neisseria gonorrhoeae* and *Bacillus subtilis*, *H. pylori* is naturally competent for DNA transformation, and diversification of *H. pylori* is likely facilitated by acquisition of genetic material from other *H. pylori* strains, or even other species that transiently colonize the gastric environment. Intragenomic recombination, facilitated by the presence of extensive non-randomly distributed DNA repeat sequences, is frequent in *H. pylori* and results in deletion or duplication of intervening DNA segments [9].

Both antigenic variation and phase variation are capable of generating extensive phenotypic diversity. DNA sequence repeats have been identified within the coding region of nearly 50 genes, which suggests that phase variation may be common in *H. pylori* (Fig. 1). This suggestion is supported by the observation that repeat sequences in several genes vary in length from strain to strain [10]. Interestingly, not all of these genes are present in all of the strains, nor are repeats always present. Therefore, variability exists across strains, not only in the length of the simple sequence repeats, but also in the presence of the repeats and even the presence of the candidate phase-variable genes. Repeat sequences are also located in the promoter region of several genes (Fig. 1) and may mediate phenotypic variation at the transcriptional level, as has been described for the class 1 outer membrane protein of *N. meningitidis* [11]. Interestingly, approximately one-half of these "contingency" genes encode surface structures that, if differentially expressed, would alter the appearance of the organism to the host immune system.

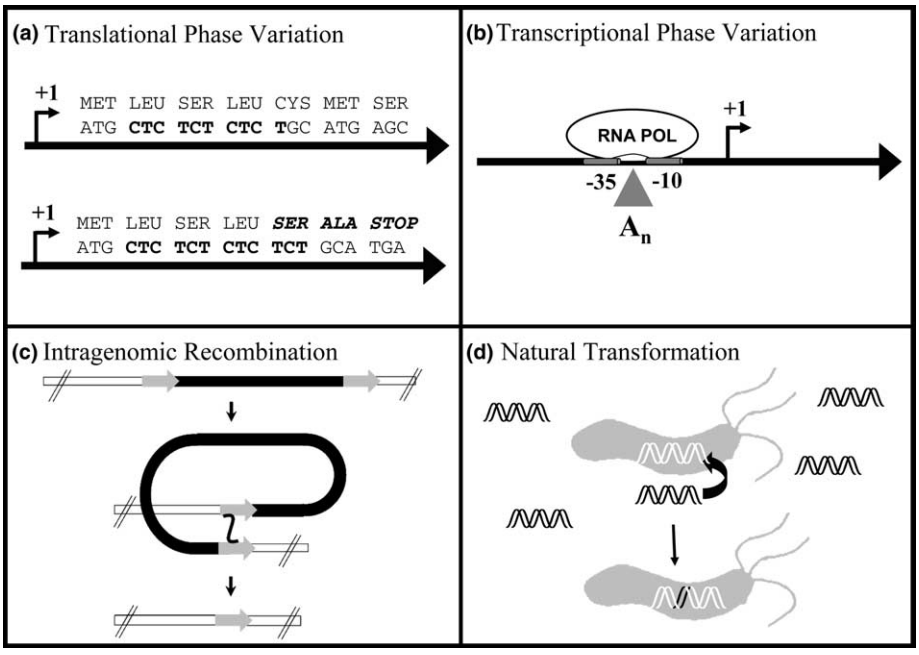

Fig. 1. Some mechanisms to generate diversity in *H. pylori*. (a) Translational phase variation may reversibly switch gene expression on and off via insertion and deletion of DNA repeat sequences (bold CT repeats) in the coding region of a gene that result in shifting the codons in and out of frame. Frameshift mutation ultimately results in the formation of a premature stop codon. (b) Transcriptional phase variation may reversibly modulate the level of gene expression via insertion and deletion of DNA repeat sequences (A_n) in the promoter region of a gene, which disrupts interactions between promoter DNA and RNA Polymerase. (c) Intragenomic recombination between repetitive DNA sequences (shaded arrows) may facilitate deletion of DNA sequences (black segment). In addition, intragenomic recombination can result in duplication of DNA sequences or formation of chimeric DNA sequences (not shown). (d) Natural transformation of *H. pylori* with extracellular DNA and subsequent recombination may introduce additional DNA sequences to the recipient and diversify its genetic repertoire.

2.2. Development of diversity in vivo

H. pylori may be considered to form a fluid population of phenotypic variants able to rapidly adapt to changing conditions within the host gastric environment. One can then imagine different scenarios by which diversity might develop (Fig. 2). For example, transmission to a new host may be clonal, or polyclonal, followed by incremental development of diversity and selection as the organism adapts over the lifetime of the new host. In this case, one would expect to find important differences among the *H. pylori* populations that are present at different points in time. Alternatively, perhaps selection acts rapidly shortly after transmission to a new host, which then harbors a population that is diverse with respect to strains from other hosts, but is itself relatively stable over time. Since *H. pylori* transmission occurs predominantly in childhood, while strains are typically recovered in adults, in this case one would see marked diversity among *H. pylori* strains from different hosts, but not within a single host at different points in time.

Although there are few data available to examine these or other possibilities, there have been occasions in which *H. pylori* strains have been isolated from the same individual several years apart. Israel et al. [12] cul-

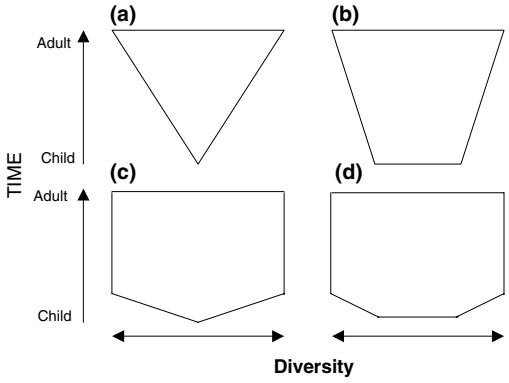

Fig. 2. Schematic diagram to illustrate different scenarios by which *H. pylori* genomic diversity might develop from the time (*y*-axis) of initial infection in childhood over the lifetime of the host. Extent of diversity (*x*-axis) is shown by the width of each figure at any point in time and increase in diversity is shown by increasing width over time. If transmission to a new host is clonal, with incremental increase in diversity over time (a), then one might identify genomic changes when comparing *H. pylori* diversity from strains isolated at any two time points that are sufficiently separated. A similar situation would occur if transmission were polyclonal (b), though diversity would be greater at early time points and change more slowly. On the other hand, if diversity develops only very early after transmission of a clonal (c) or polyclonal (d) infection, then comparison of strains isolated at different times during adulthood would not likely show greater diversity across than within time points.

tured *H. pylori* from the source patient of the completely sequenced J99 strain six years after the original isolate was obtained. Randomly amplified polymorphic DNA (RAPD) PCR showed that single colony *H. pylori* isolates were closely related to the archival J99 strain, indicating that the patient was colonized with a single strain at both time points. However, subtle differences were noted and higher resolution analysis by whole genome DNA microarray demonstrated that all of the isolates were genetically distinct variants that differed overall in about 3% of the J99 loci. Unfortunately, since only a single archival strain was available, it was impossible to determine if this diversity was present at the time the original isolate was obtained, or if it had developed over the subsequent six years. This limitation is emphasized by a recent analysis of multiple colonies isolated nine years apart from two patients [13], which suggested that the diversity among colonies isolated from each patient within a time point was as great as that found across time points. An alternative strategy is to examine diversity in paired pools of isolates obtained from patients at different times, which has been interpreted to reflect in vivo genetic drift of the population over time [14].

The different sampling approaches used in these studies each have limitations for assessment of the development of *H. pylori* diversity over time. The single colony approach allows the recognition of mixed colonization with different strains and changes in individual strains over time, but it is susceptible to sampling errors. The pooled colony approach may be more representative of the population and reflect differences at the population level, but fails to recognize mixed colonization and changes that occur in individual strains over time. Thus, the question of to what extent and at what rate diversity develops during chronic *H. pylori* infection remains unresolved.

3. How might bacterial diversity promote chronic infection?

Long-term persistence as an extracellular pathogen in the gastric environment requires that *H. pylori* respond and adapt to continuous immune and inflammatory responses in addition to other host physiological changes. Multiple strategies have evolved within bacteria to sense and respond to changing environmental conditions, including two-component regulatory systems and global regulatory proteins. However, similar to other bacterial species that occupy a restricted host niche, such as *Haemophilus influenzae* and *N. gonorrhoeae*, *H. pylori* has a relatively small genome that has retained only a limited repertoire of classical regulatory elements [15]. For example, the *H. pylori* genome is approximately one half the size of the *Escherichia coli* genome, yet encodes only about one third the proteins involved in two-component regulatory systems [16]. In the absence of multiple regulatory elements, *H. pylori* may employ an alternative adaptive strategy in which persistence is achieved by continuous diversification and selection of the fittest individuals in each microniche within the gastric environment. This adaptive strategy may be exploited to facilitate persistence by generating diverse phenotypic variants that promote evasion of host immunity, modulate adhesion, and modify the bacterial-host interaction.

3.1. Evasion of host immunity

One of the paradoxes of *H. pylori* pathobiology is that the organism is capable of long-term persistence in the gastric mucosa despite not only a variety of innate host defenses, but also an adaptive humoral and cellular immune response that is specific to bacterial epitopes. Antigenic and phase variation may contribute to the ability of *H. pylori* to persist despite the host immune response. Both antigenic variation, in which combinatorial DNA shuffling creates antigenically distinct proteins, and phase variation, in which reversible DNA sequence changes result in on/off switching of gene expression, are capable of generating extensive phenotypic diversity.

3.1.1. Antigenic variation

The hypothesis that antigenic variation plays a role in immune evasion is perhaps best studied with *Borrelia* species. *Borrelia* spp., including *B. recurrentis* and *B. hermsii*, are associated with relapsing fever that corresponds with recurring waves of spirochetemia. Each wave of spirochetemia is associated with the emergence of a novel antigenic variant that is subsequently cleared by a specific humoral response, only to be followed by another wave of spirochetemia as yet another antigenic variant emerges. A specific immune response is directed against the variable major proteins (VMPs), a family of surface-exposed lipoproteins that are encoded by the *vmp* genes. *B. hermsii* encodes up to 40 *vmp* gene cassettes, but only a single locus is expressed at any one time. Antigenic variation is due to a non-reciprocal recombination event that occurs between a silent *vmp* gene cassette and a unique *vmp* expression site, resulting in expression of different VMPs over time.

By analogy, it is plausible that antigenic variation in *H. pylori* also functions to avoid host immunity. Although experimental evidence is modest, the case is probably strongest for *cagY*, a homolog of the *Agrobacterium tumefaciens* VirB10 protein, which coats the type IV secretion system pilus that is required for translocation of CagA into gastric epithelial cells. The surface-exposed CagY is subject to antigenic variation that results from recombination between direct DNA repeats.

The *cagY* gene contains a remarkable number of direct DNA repeats, and the DNA rearrangements that occur from the recombination events among the repeat sequences result in the expression of continuously changing proteins that vary in size and antigenicity [17]. Interestingly, bacterial populations from the same host contain strains that express CagY of various sizes. However, since studies to date have failed to detect an immune response to CagY, its role in immune evasion remains speculative. The role of recombination in *H. pylori* persistence has recently been suggested by the observation that an isogenic mutant defective in homologous recombination is cleared from mice more effectively than wild type, apparently via a more aggressive Th-1 type immune response [18].

3.1.2. Phase variation

The link between phase variation and immune evasion is an attractive hypothesis for which there is surprisingly little direct experimental evidence. The clearest example is the use of phase variation by *Salmonella enterica* serotype Typhimurium to avoid cross-immunity to common fimbrial antigens among *Salmonella* serotypes. Immunization of mice with fimbriated *S. typhimurium* (phase-on variant) results in selection against fimbriated variants upon subsequent challenge with virulent *S. enteritidis*. Vaccination of mice with *S. typhimurium* does not confer protection against challenge with *S. enteritidis*, presumably because phase-off variants are able to evade cross-immunity [19]. This evasion does not occur if phase variation is prevented in the *S. enteritidis* challenge strain [20].

A considerable potential for phenotypic diversity in *H. pylori* lies in the presence of a large family of polymorphic outer membrane proteins (OMPs), many of which are subject to phase variable expression. Members of this OMP family have been identified as adhesins and porins, but the majority of the OMPs remain uncharacterized. Due to extensive 5′ and 3′ homology, it has been proposed that recombination among these paralogous genes could result in the production of many different mosaic genes and variable proteins [16]. Modification of OMP gene expression has recently been demonstrated in vivo during experimental infection of rhesus macaques [21]. Microarray analysis demonstrated that the gene encoding the ABO blood group antigen-binding adhesin, *babA*, was deleted in isolates of *H. pylori* strain J166 recovered after infection of the rhesus macaque. In some isolates, the *babA* gene was replaced by a duplicate copy of *babB*, a paralogue of unknown function. In other isolates *babA* was not expressed due to variation in the number of dinucleotide CT repeats in the 5′ coding region of the gene. This study demonstrated the occurrence of both antigenic and phase variation of *H. pylori* OMPS within a host, but it remains unclear whether passage through the rhesus macaque selected for deletion of *babA*, duplication of *babB*, or both. It is possible that loss of *babA* reflects antigenic variation that the bacterium uses to avoid the host immune response. However, as is the case for CagY, neither BabA nor BabB have been demonstrated to be immunodominant antigens in humans or rhesus macaques. Alternatively, BabB may function as an adhesin and overexpression of BabB may be advantageous for persistence in the rhesus macaque. Diversity in *H. pylori* OMP expression may contribute to *H. pylori* persistence by altering adhesion to different receptors present in different hosts, or even in different microenvironments within a host.

3.2. Modulation of adhesion

The diverse repertoire and genetic plasticity of OMPs may facilitate persistence by allowing *H. pylori* to engage in a broad range of interactions with host receptors. *H. pylori* adherence is multifactorial and the adhesin–receptor interactions may vary not only across individuals, but also over the course of infection within a single individual (Fig. 3). On the one hand, adherent bacteria avoid being washed into the lumen of the stomach and may gain access to nutrients by delivering toxins and other effector molecules that damage gastric epithelial cells. On the other hand, these adherent cells might stimulate the host response and induce inflammation. Thus, the *H. pylori* population may experience cycles of selection for adherent or non-adherent cells depending on nutrient levels and the host immune response.

The ABO blood group antigen-binding adhesin, BabA, is perhaps the best-studied adhesin in *H. pylori*. Initial observations suggested that BabA binds specifically to Lewis b (Leb) and fucosylated H1 antigens expressed by gastric epithelial cells [22]. However, it was recently reported that BabA adhesins from strains isolated in different geographic regions have a broad range of binding specificities that include the ALeb and BLeb antigens, in addition to the Leb antigen [23]. It has been proposed that these binding specificities reflect strain adaptation to different glycosylation patterns that predominate in local populations. For example, the strains that bind only Leb and fucosylated H1 antigens (the blood group antigens that define the blood group O phenotype) are considered ‘‘specialist’’ strains and are commonly found in South American populations, which are unique in being almost entirely of blood group O phenotype. Strains that bind ALeb and BLeb antigens, in addition to Leb, are considered ‘‘generalist’’ strains, and their broad distribution reflects the abundance of A, B, and O blood groups in most human populations.

BabA adhesins from strains around the world also display a broad range of binding affinities that vary from a 300-fold range in ALeb affinities to a 1500-fold range

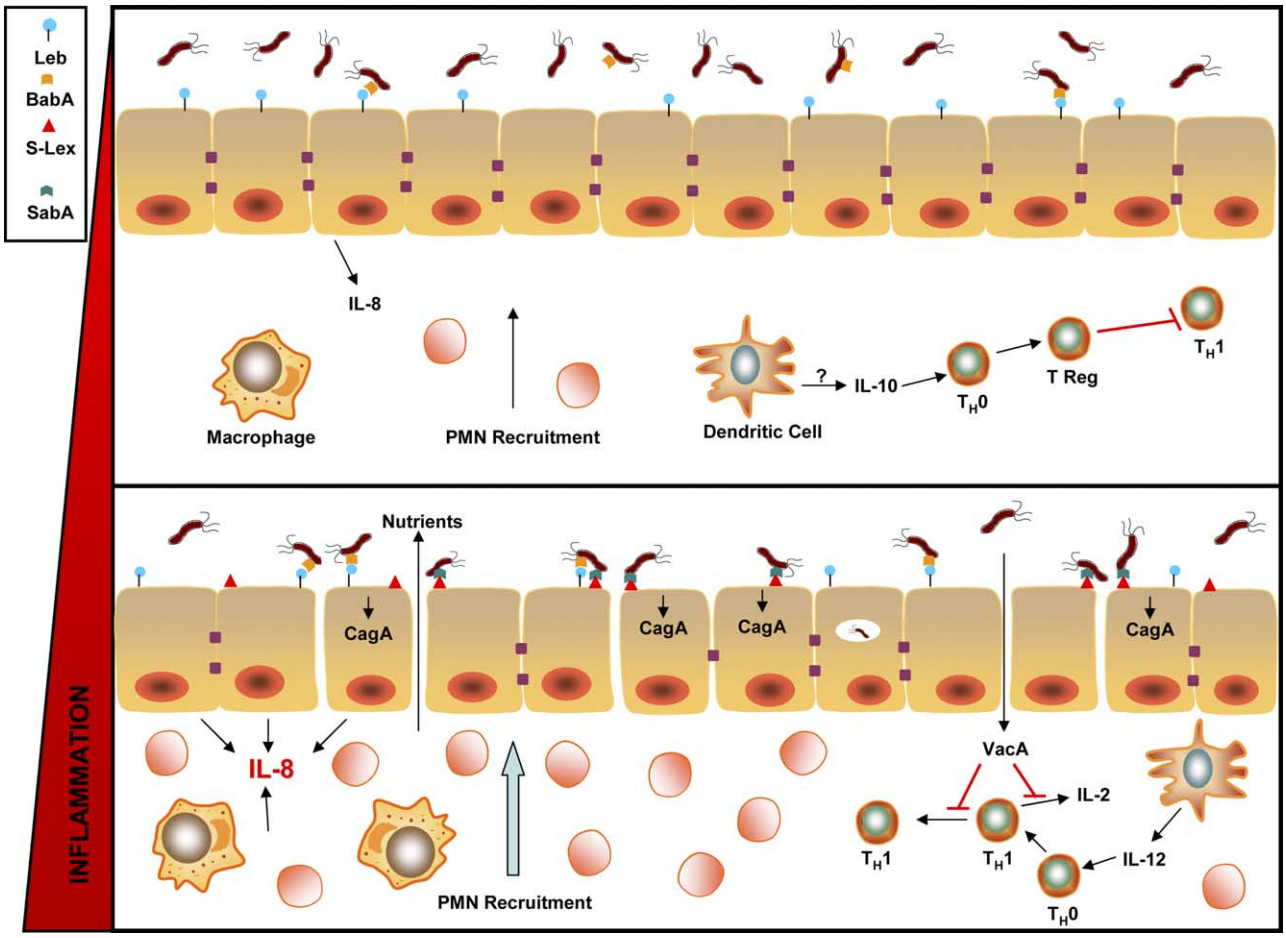

Fig. 3. Schematic diagram showing the interaction between *H. pylori* and the host under conditions of low and high inflammation (gastritis). Although depicted as two extremes, inflammation is probably best thought of as a continuum that may differ between anatomic regions of the stomach and may cycle from one extreme to another. The upper panel reflects low inflammation conditions that may occur early in infection, during infection with Cag PAI (−) strains, and as a cyclical response of the host to high inflammation. Gastritis occurs with all *H. pylori* infections and is characterized by PMN and monocyte (macrophage) recruitment. Attachment of *H. pylori* to gastric epithelial cells is mediated by BabA and perhaps other adhesins not yet identified. The lower panel illustrates high inflammation conditions that occur during *H. pylori* infection, especially infection with CagA/VacA/BabA+ (triple positive) strains. Under high inflammation conditions, sialyl-Lex (S-Lex) is upregulated and serves as a receptor for the *H. pylori* SabA adhesin. This additional adhesin-receptor interaction between S-Lex and SabA is thought to mediate a more intimate interaction and thus facilitate closer attachment of *H. pylori* to the epithelium. Translocation of CagA into host epithelial cells leads to breakdown of tight junctions between gastric epithelial cells and a leaky epithelium. Nutrients transit into the gastric mucous and *H. pylori* virulence factors, such as VacA, entering the submucosa. The lower right section of the high inflammation panel describes the effects of VacA on T cells that have been recruited to the gastric submucosa. VacA inhibits IL-2 production and blocks T cell proliferation, which might otherwise lead to *H. pylori* eradication. In order to persist in gastric epithelium, *H. pylori* has evolved mechanisms that are not yet fully understood to promote the T regulatory cell response. As shown in the upper panel, Treg cells are derived from Th-0 cells under the influence of IL-10. Treg cells dampen the immune response by blocking both CD4+ and CD8+ effector T cell functions.

in Leb affinities. Phylogenetic analysis of the variable central region of *babA* sequences from 66 clinical isolates revealed that differences in specificity and binding affinity could each result from subtle amino acid differences [23]. Therefore, point mutation or recombination between short stretches of *babA* sequences can result in BabA adhesins with different binding strengths and specificities, and strains with optimal adherence phenotypes in an individual will be selected.

Changing environments within an individual host over time may also select for different adherence qualities in the *H. pylori* population. Chronic *H. pylori* infection changes the gastric environment by inducing inflammation that raises gastric pH and causes hypochlorhydria in some patients. However, the inflammation that *H. pylori* provokes actually upregulates the expression of sialylated glycoconjugates that can participate in adhesin–receptor interactions with the *H. pylori* adhesin, SabA [24]. The interaction of SabA with the sialyl-dimeric-Lewis × antigens expressed on membrane glycosphingolipids is weaker but more intimate than the interaction between BabA and the Leb antigen, which is expressed on glycoproteins that protrude from the cell. It has been proposed that the phase-variable

expression of SabA may allow intimately adhered cells to escape from sites of vigorous host responses [24].

With only a limited repertoire of classical regulatory elements, *H. pylori* appears to use genetic diversification to modulate adherence. Several members of the major OMP family display phase variable expression, which allows diversification of the *H. pylori* population into a mixture of phase-on and phase-off variants. Recombination may also modulate *H. pylori* adherence. Duplication of *babA* or *babB* and deletion of its paralogue have both been reported [21,25]. In the case of *babA* duplication, some human strains possess silent *babA* sequences and harbor occasional cells within the population that have undergone a recombination event between the silent *babA* and *babB*, thus activating BabA expression and Leb binding. In the event that binding Leb becomes favorable to the *H. pylori* population, the BabA-expressing cells will be available for selection and proliferation. Alternatively, passage of *H. pylori* strain J166 through rhesus macaques selected for bacteria that had a duplicate copy of the *babB* gene in the *babA* locus and thus did not bind Leb.

We and others have hypothesized that both phase variation and recombination contribute to the dynamic adherence properties of *H. pylori* by producing a heterogeneous population that includes some cells with the ability to attach to the gastric epithelium and others that remain unattached and escape inflammation. The proportion of adherent and non-adherent cells is selected in each host environment, and this diversity leads to persistence of the *H. pylori* population by ensuring that a population with optimal adherence qualities will emerge in each environment. However, there is probably genetic diversity even within the population of adherent cells that results in phenotypic variants that differ substantially in their interaction with the host. This diversity provides yet another tier of host selection that contributes to modulation of bacteria–host interactions.

3.3. Modulation of bacteria–host interactions

Once attached, *H. pylori* can engage in a broad range of interactions with gastric epithelial cells that is largely determined by the presence of bacterial virulence factors. For example, about 60% of *H. pylori* strains in the United States and Europe carry the 37 kb Cag pathogenicity island (PAI), which is more commonly found in patients with peptic ulcer disease or gastric cancer than in those that are asymptomatically infected. Six of the 27 genes on the PAI are homologous with components of the type IV secretion system that was originally described in *Agrobacterium tumefaciens* but is now known to be present in many human pathogens, including *Legionella pneumophila*, *Bordetella pertussis*, *Brucella*, and *Bartonella*. Strains that carry the PAI typically can induce interleukin-8 (IL-8) in gastric epithelial cells via Nod1 recognition of bacterial peptidoglycan delivered into host cells by the Type IV secretion apparatus [26].

H. pylori virulence factors vary not only in their presence within a strain, but they also display extensive allelic diversity across strains. Low resolution methods such as restriction fragment length polymorphism (RFLP) and RAPD may sometimes identify multiple strains within a single host. However, it is likely that extensive variability is common within a strain, and in fact clonal *H. pylori* infection may well not exist. A good example of this diversity is the CagA protein, which is an effector known to be secreted by the Type IV system in *H. pylori*. Once translocated into gastric epithelial cells, CagA is tyrosine phosphorylated by host kinases, after which it interrupts host signal transduction and induces a rearrangement of the actin cytoskeleton. The 3′ portion of *cagA* contains a series of direct DNA repeats. Intragenomic recombination between these repeats results in deletion or duplication of tyrosine phosphorylation sites in CagA. Isolates from the same individual have been shown to harbor *cagA* genes that differ in size due to the presence of different numbers of DNA repeats and tyrosine phosphorylation motifs. The addition or deletion of tyrosine phosphorylation motifs in the CagA protein results in differential phosphorylation of CagA in the host cell, which is associated with the degree of disruption in host cell morphology and the development of gastric cancer [9]. It seems likely that *H. pylori* can respond to selective pressure by increasing or decreasing the number of tyrosine phosphorylation motifs within the population in any given individual, thereby modulating the extent of the host-bacterial interaction.

Another *H. pylori* protein that interacts with host gastric epithelial cells is the vacuolating cytotoxin, VacA, which is a pore-forming toxin that causes epithelial cell vacuolation. There is extensive diversity among VacA cytotoxins across strains due to the mosaic nature of the *vacA* gene. *vacA* varies most strikingly in its midregion, which encodes the toxin-cell binding domain. The type m1 VacA binds more extensively to the host cell and is more closely associated with disease than is type m2. *vacA* also varies in its signal region, which encodes the signal peptide and the N terminus of the mature toxin. The type s1 VacA is toxic, but type s2 VacA has a short N terminal extension on the mature toxin that eliminates vacuolating activity. Strains with all four combinations of the *vacA* signal and midregions have been found, implying previous homologous recombination within *vacA* among *H. pylori* strains. Mixed infection within one stomach (i.e., different DNA fingerprints) with strains carrying different *vacA* alleles has been reported, and so too has the culture of isolates with the same DNA fingerprint having different *vacA* alleles. The sequences of the *vacA* alleles suggest recent homologous recombination, which has resulted

in closely related strains that possess different VacA proteins and thus different toxicity [27]. Thus VacA as well as CagA, and probably other proteins as well, can demonstrate marked diversity that modulates the host-bacterial interaction.

H. pylori strains that carry the *vacAs1* allele and CagA (Type I strains) are more often found in patients with ulcer or gastric adenocarcinoma, but the so-called "triple positive" strains that express *vacAs1*, *cagA*, and *babA* are even more strongly associated with disease [28]. This finding suggests that adherence of *H. pylori* to gastric epithelial cells via the BabA adhesin may promote disease through the efficient delivery of bacterial toxins or other effectors. Put differently, since *vacA* s1, *cagA*, and *babA* are often co-expressed though genetically unlinked, they likely serve a common function that results in their selection. This is consistent with the recent observation that in humans with mixed infection (PAI+ and PAI−), *cagA* – strains are preferentially localized to the mucous gel or in areas near the apical surface of epithelial cells, while *cagA*+ strains are seen in more intimate contact with the epithelium [29]. This pattern of colonization may be due to the frequent presence of the BabA adhesin in strains with the PAI.

4. *H. pylori* persistence and host immunity

The hallmark of *H. pylori* infection is inflammation in the gastric mucosa, termed chronic active gastritis. The cellular infiltrate of *H. pylori* gastritis consists largely of polymorphonuclear leukocytes (PMNs), plasma cells, and Th-1 biased lymphocytes, predominantly CD4+ T cells that secrete interferon gamma (IFN-γ) and other pro-inflammatory cytokines. Since *H. pylori* is primarily an extracellular infection, this bias toward cellular immunity at first seemed puzzling, and perhaps maladaptive for host defense, since IgA and other immunoglobulins are considered most important for control of mucosal pathogens. It was reasoned that bacterial persistence resulted from the failure of an adequate humoral immune response, and that if this could be overcome with vaccines or other immunomodulators, perhaps *H. pylori* infection could be prevented or more effectively treated. But recent converging evidence suggests that the host cellular immune response is not so much maladaptive as it is inadequate. In mouse models of *H. pylori* there is generally an inverse relationship between the extent of bacterial colonization and the degree of gastritis. Furthermore, host immunity fails to clear infection in normal mice challenged with *H. pylori*, but enhanced gastritis induced by immunization [30] or by transfer of normal donor splenocytes to SCID mice [31] is sometimes sufficient to clear infection. On the other hand, humoral immunity seems to be relatively less important for the outcome of *H. pylori* infection, at least in mouse models, since immunization can induce protective immunity in B cell knockout mice that is equivalent to that in wild type mice [32].

Chronic gastritis is a consistent but ultimately ineffective host response to *H. pylori* infection. Indeed, we speculate that some inflammation may be essential for chronic *H. pylori* colonization. Damage to the gastric epithelium from inflammatory mediators may improve the nutritional environment for bacterial cells lying close to the epithelial layer. Attachment of *H. pylori* to the gastric epithelium also appears to be mediated in part by attachment to sialyl-Lewis × antigens that are induced by inflammation, a form of selectin mimicry. Thus, from the bacterial point of view, the relationship between *H. pylori* and gastritis may be akin to the fairy tale of Goldilocks and the Three Bears: the proper amount of gastritis to promote chronic infection may be not too little and not too much, but just right. Viewed in this way, it is not surprising that *H. pylori* has evolved several mechanisms by which the innate and adaptive arms of the immune system are tightly regulated, which is probably critical to persistent infection (Fig. 3).

4.1. Innate immunity

The innate immune response includes both physical and chemical barriers that are basic components of gastric physiology, as well as cells and cellular products that act to eliminate pathogens and stimulate the adaptive immune response. Here we focus on three mechanisms by which *H. pylori* modulates innate immunity: evasion of bacterial pattern recognition, avoidance of phagocyte killing, and invasion of host cells.

4.1.1. Evasion of pattern recognition

Eleven different mammalian Toll-like receptors (TLRs) are now recognized, which respond to essential conserved microbial components called pathogen associated microbial patterns (PAMPs). These include TLR4 (bacterial lipopolysaccharide, LPS), TLR2 (bacterial lipoproteins), TLR3 (double-stranded RNA), TLR5 (flagellin), TLR7/8 (single stranded RNA) and TLR9 (CpG motifs). Recognition of pathogens by TLRs on host cells has complex effects that act via the innate immune system but also by priming of the adaptive immune response.

H. pylori is capable of subverting at least portions of the host TLR pattern recognition response and perhaps exploiting others. For example, *H. pylori* FlaA and FlaB are not recognized by cultured gastric epithelial cells expressing TLR5 [33], which typically responds to bacterial flagellin by induction of the pro-inflammatory chemokine, IL-8. *H. pylori* also differs from other Gram-negative bacteria in the TLR4-mediated inflammatory response to LPS. It has long been recognized that *H. pylori* LPS is 100–10,000-fold less potent than that

of *E. coli* and other Gram-negative bacteria. This appears to be due to the very weak agonist activity of intact *H. pylori* LPS for TLR4, which is expressed on gastric epithelium [34].

TLR signals are important not only for the initial pro-inflammatory response to infection, but also for activation of compensatory anti-inflammatory mechanisms such as production of IL-10 and recruitment of regulatory T cells [35]. Recent evidence in TLR biology suggests that microbial pathogens have adapted to exploit this anti-inflammatory response. For example, TLR2 has been identified as the major receptor for PAMPs of gram-positive bacteria, but it also induces immunosuppression and bias toward a Th-2 immune response. TLR2 recognizes intact *H. pylori* but is not expressed on the gastric epithelium. Therefore, *H. pylori* may promote persistent infection by colonizing a TLR2 deficient environment on the one hand, and then exploiting TLR2 signaling for immune suppression after recruitment of infiltrating blood leukocytes (PMNs and monocytes) that do express TLR2 [34]. A TLR2-mediated anti-inflammatory response would likely further promote *H. pylori* persistence.

Another class of pathogen recognition receptors are the C-type lectins that recognize specific carbohydrate structures present on the cell wall of pathogens. One of these lectins expressed on dendritic cells is DC-SIGN (dendritic cell-specific intercellular adhesion molecule-grabbing non-integrin), which has a particularly high affinity for Lewis blood-group antigens that contain fucose. The unifying feature of pathogens that bind DC-SIGN is that they cause chronic infections whose persistence is sensitive to the relative balance of Th-1 and Th-2 immunity. Recent evidence suggests that decoration of *H. pylori* LPS with Lewis blood group antigens mediates binding to DC-SIGN on gastric dendritic cells and blocks development of a Th-1 response [36]. Lewis antigen expression in *H. pylori* is phase variable by translational frame shifts in glycosyltransferase genes that occur during replication. Therefore, depending upon the extent of inflammation at any time and its relative positive and negative selective effects, the optimal population of Lewis antigen expressing *H. pylori* cells might be selected to promote persistence and chronic infection.

4.1.2. Evasion of phagocyte killing

H. pylori chronic gastritis is characterized by a neutrophilic infiltrate, which is unusual because PMNs more commonly represent only the acute inflammatory response that is later replaced by adaptive immune cells and macrophages. Chronic maintenance of a neutrophilic response is in part due to the direct recruitment of PMNs by *H. pylori* through two independent mechanisms. Cag PAI+ *H. pylori* induces gastric epithelial cells to produce IL-8, a neutrophil chemotaxin. In addition, *H. pylori* produces neutrophil activating protein (NapA), which induces chemotaxis of monocytes and neutrophils by upregulation of β_2 integrins. Although not as numerous as neutrophils, macrophages are also part of the inflammatory infiltrate recruited to the gastric mucosa during *H. pylori* infection.

If inflammation may in part be adaptive and perhaps essential for persistent *H. pylori* infection, perhaps via its effects on host epithelial cell integrity, then the bacterium must have strategies to persist in a phagocyte-rich environment. There are no doubt many mechanisms by which this occurs, and here we consider three. First, *H. pylori* broadly inhibits the phagocyte function of monocytes and PMNs by a mechanism involving the Type IV secretion system encoded on the Cag PAI [37]. The Cag PAI may also mediate delayed entry into macrophages and fusion of phagosomes into a structure that contains multiple organisms that are viable for up to 24 h [38]. However, these observations require confirmation since they are based on comparison between Type I (Cag PAI+) and Type II (Cag PAI−) strains that are not isogenic. Second, *H. pylori* induces a mitochondrial cell death pathway to promote macrophage apoptosis [39]. Finally, *H. pylori* has strategies to blunt the toxic effects of extracellular products of phagocytes. These include not only catalase, superoxide dismutase, and other well-recognized mechanisms for protection against oxidative stress, but also elegant interventions in the nitric oxide (NO) pathway, which is a central component of innate immunity and an effective antimicrobial agent. For example, NO production is regulated by mammalian arginases that compete with NO synthases for the common substrate, L-arginine. *H. pylori* arginase encoded by the *rocF* gene can reduce NO production, both by substrate competition [40] and by inhibition of inducible NO synthase (iNOS) translation by spermine, a downstream product of the conversion of arginine to ornithine [41]. *H. pylori* also encodes AhpC, a member of the bacterial peroxiredoxin family that protects against oxidation of DNA and other molecules by peroxynitrite, a product of NO and O_2^- [42].

4.1.3. Epithelial cell invasion

H. pylori is considered primarily a mucosal pathogen. However, for nearly 20 years, almost from the time the organism was first described, there have been scattered but persistent reports of apparent bacterial invasion in human gastric biopsies and in cultured epithelial cells (reviewed in [43]). The evidence is probably sufficient to conclude that *H. pylori* can invade epithelial cells, though the primary niche is the gastric mucous layer and perhaps only 10% or fewer bacterial cells are intimately associated with the epithelium. Time-lapse microscopy suggests that, at least in vitro, intracellular bacteria are viable and can repopulate the extracellular environment [44]. Transient cellular invasion by a subpopulation of *H. pylori* might therefore be a strategy

for persistence in the face of unfavorable conditions, such as gastric acid, antibiotics, or the host inflammatory response. Recent elegant experiments suggest that *E. coli* cystitis, also a predominantly mucosal infection, may exploit a similar strategy [45]. Video microscopy showed that uropathogenic *E. coli* can invade bladder epithelial cells in a FimH-dependent manner, followed by organization of coccoid bacteria into a tightly packed cytoplasmic matrix termed a ''pod''. The impermeable uroplakin shell of the pod is thought to provide a barrier to antibiotics and perhaps also the host inflammatory response, which might explain in part the vexing problem of recurrent cystitis. Invasion of host epithelial cells to promote chronic infection may in fact be a common strategy for mucosal pathogens previously thought to have a uniformly extracellular lifestyle.

4.2. Adaptive immunity

Although the adaptive immune response is essential to host survival, so too is its regulation in order to prevent severe collateral damage. Recent evidence suggests that *H. pylori* modulates adaptive immunity, both by exploiting host regulatory T cells and by interfering directly with T cell proliferation.

4.2.1. Regulatory T cells

The study of suppressor T cells first proposed in the 1970s has recently been reinvigorated with the identification of two populations of regulatory T cells, inducible and naturally occurring. Inducible (adaptive) regulatory *T* cells include CD4+ cells that secrete IL-10 (T_R1) or TGF-β (T_H3), as well as CD8+ T cells. Naturally occurring (constitutive) regulatory T cells, which express CD4 and high levels of CD25, can suppress proliferation and cytokine production of both CD4+ and CD8+ T cells via a cell-cell contact mechanism that is dependent upon expression of the transcription factor FOXP3.

A series of recent studies has demonstrated a role for CD4+CD25+ regulatory T cells in suppressing the immune response to *H. pylori* in the gastric mucosa. The initial observation was the paradoxical finding that T cells from *H. pylori*-uninfected individuals are equally or perhaps even more reactive to *H. pylori* antigens than are T cells from infected individuals. Lundgren et al. [46] suggested the hypothesis that repetitive stimulation of T cells from infected persons by *H. pylori* antigens might induce *H. pylori*-specific regulatory T cells. They showed that the unresponsiveness of memory T cells from infected individuals could be abolished by depletion of CD4+CD25^{high} regulatory T cells. This immune suppression was *H. pylori* specific, since stimulation with tetanus toxoid induced comparable responses in memory cells from infected and uninfected persons in both the presence and absence of CD4+CD25^{high} regulatory T cells. These in vitro observations were quickly extended to mice. Reconstitution of athymic mice with lymph node cells depleted of CD25+ T cells showed increased gastritis and reduced colonization after challenge with *H. pylori*, compared to mice that received undepleted lymph node cells [47]. Furthermore, *H. pylori*-infected individuals have more CD4+CD25^{high} T cells expressing FOXP3 in the gastric mucosa than uninfected individuals [48], which is consistent with the hypothesis that regulatory T cells may suppress mucosal immune responses and contribute to the persistence of *H. pylori* infection. These observations can be placed in the broader context of data suggesting that both inducible and naturally occurring regulatory T cells are exploited by a broad range of microbial pathogens [49].

4.2.2. Inhibition of T cell proliferation

In addition to controlling the inflammatory response by subverting host regulatory T cells, *H. pylori* also interferes directly with T cell proliferation via VacA. Several mechanisms appear to be involved [50–52]. First, VacA self-assembles to form hexameric, anion-specific channels in cell membranes. For activated T cells, VacA channels interfere with Ca2+ signaling and nuclear localization of the transcription factor NFAT, abrogating IL-2 transcription. This effect has been likened to the immunosuppressive action of cyclosporin and FK506. VacA also inhibits T cell activation downstream of IL-2 by inhibiting IL-2-driven proliferation. This effect of VacA resembles the actions of two other immunosuppressive drugs, rapamycin and sanglifehrinA. Finally, a third effect of VacA involves binding of an unknown receptor on T cells, leading to Rac/p38 activation, actin rearrangement, and inhibition of T cell proliferation. Since *H. pylori* can disrupt epithelial cell tight junctions, VacA might act as a distant effector that can reach the lamina propria and block proliferation of T cells in the local gastric environment. These observations might explain in part the finding that *H. pylori* with a null mutation in *vacA* is compromised in its ability to establish infection in a mouse model [53].

5. Conclusions

H. pylori has evolved to persistently colonize its human host for at least tens of thousands of years, and perhaps longer. The result of this co-evolution has been a détente; disease, when it occurs, may be viewed as a result of ''inconclusive negotiations for symbiosis, an overstepping of the line by one side or the other, a biologic misinterpretation of border'' [54]. To maintain persistent infection, *H. pylori* has evolved strategies that we have categorized, somewhat arbitrarily, into bacterial diversity and evasion of host immunity. The view that emerges from this analysis is that the bacterial-host

interaction is a complex biological system. *H. pylori* cannot be viewed simply as, for example, pro-inflammatory or anti-inflammatory, adherent or non-adherent, pro-apoptotic or anti-apoptotic. Instead, *H. pylori* modulates its interaction with the host in order to promote chronic infection, and uses diverse mechanisms to do so. Recent controversial evidence suggests that while chronic *H. pylori* colonization causes some diseases, it may protect against others that are becoming more common in developed countries where *H. pylori* prevalence is declining, such as gastrointestinal reflux and adenocarcinoma of the distal esophagus [55]. That gastric *Helicobacter* infection is ubiquitous in the animal kingdom reinforces this view and reminds us that infection may have benefits as well as costs. Ultimately, understanding *H. pylori* and the biology of persistent infection may not only suggest strategies for treatment and prevention, but perhaps even lead to more sophisticated approaches that can be used to shift the host–pathogen interaction even more toward commensalism.

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